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13. ABSTRACT

The toxic marine saponin holothurin A from the Bahamian sea cucumber has been studied with regard to potency in blockade of responses of phrenic nerve-diaphragm (PN-D) preparations from the rat and the guinea pig under the additional stresses of high environmental pressure or variable loading of the muscle. In the rat tissue, the toxin ($H^-$) and its desulfated derivative (DeH) afford a contrast in sensitivity to the effects of elevated pressure: (a) the potency of $H^-$ at $1.0 \times 10^{-4}$ M is quite high at 14.7 psia, falls sharply as pressure is increased to 130 psia, and then increases moderately as pressure is further raised toward 330 psia; (b) the effect of increased pressure on DeH potency is only marginal. At ambient pressure, the effectiveness of $1.0 \times 10^{-4}$ $MH^-$ in depressing twitch responses of rat or guinea pig PN-D preparations is quite high near zero loading of the muscle, and falls to negligible levels as the static loading factor is increased toward 10-20 g/g of tissue. These observations are interpreted in terms of a working model for toxin receptors in junctional areas of PN-D tissues.



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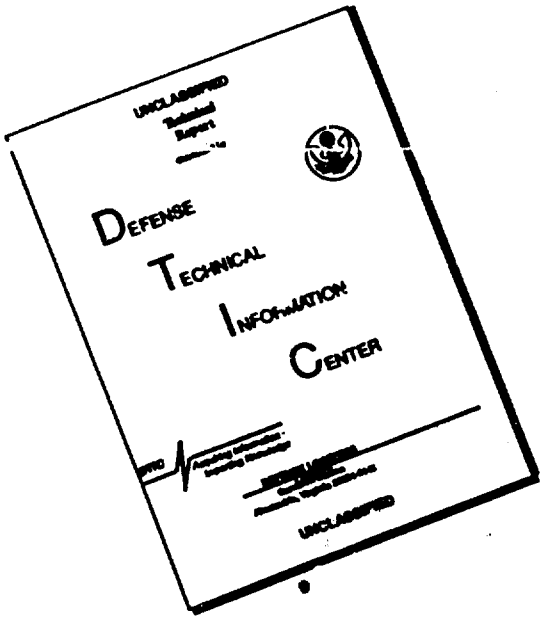


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| Pressure | | | | | | |
| Muscle Loading | | | | | | |
| Toxic Actions | | | | | | |
| Echinoderm Saponins | | | | | | |
| Holothurin | | | | | | |
| Phrenic nerve-diaphragm | | | | | | |
| Toxin receptors | | | | | | |

TOXICOLOGY AND APPLIED PHARMACOLOGY 22, 115–127 (1972)

# Effects of Pressure and Muscle Loading on the Toxic Actions of Echinoderm Saponins in Neuromuscular Tissues[1]

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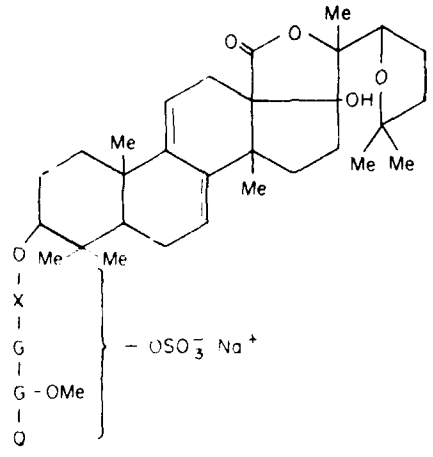
Effects of Pressure and Muscle Loading on the Toxic Actions of Echinoderm Saponins in Neuromuscular Tissues. FRIESS, S. L., DURANT, R. C., FINK, W. L., and CHANLEY J. D. (1972). *Toxicol. Appl. Pharmacol.* **22**, 115–127. The toxic marine saponin holothurin A from the Bahamian sea cucumber has been studied with regard to potency in blockade of responses of phrenic nerve–diaphragm (PN-D) preparations from the rat and the guinea pig under the additional stresses of high environmental pressure or variable loading of the muscle. In the rat tissue, the toxin (H) and its desulfated derivative (DeH) afford a contrast in sensitivity to the effects of elevated pressure: (a) the potency of H at $1.0 \times 10^{-4}$ M is quite high at 14.7 psia, falls sharply as pressure is increased to 130 psia, and then increases moderately as pressure is further raised toward 330 psia; (b) the effect of increased pressure on DeH potency is only marginal. At ambient pressure, the effectiveness of $1.0 \times 10^{-4}$ M H in depressing twitch responses of rat or guinea pig PN-D preparations is quite high near zero loading of the muscle, and falls to negligible levels as the static loading factor is increased toward 10–20 g/g of tissue. These observations are interpreted in terms of a working model for toxin receptors in junctional areas of PN-D tissues.

Recent national emphasis on the useful deployment of man at great depths in the sea for extended periods of time has led to increased attention to the toxic hazards stemming from potential envenomation by poisonous marine animals. One source of such hazards is found in the family of toxic steroidal saponin esters elaborated by members of the poisonous echinoderms, e.g., sea cucumbers and starfish. These animals can pose a threat to man, directly, through physical exposure to toxins released into sea water, or indirectly if the toxins or their derivatives should enter the endogenous food chain at depth. A major example of this class of marine toxins is afforded by the potent anionic saponin holothurin A (Nigrelli *et al.*, 1955) elaborated by the Bahamian sea cucumber *Actinopyga agassizi* Selenka. It contains as important structural units (Fig. 1) a complex steroid nucleus (Chanley *et al.*, 1966), an array of sugars attached glycosidically and a

[1] From Bureau of Medicine and Surgery, Navy Department, Research tasks MR041.06.01-0006B and M4306.02-3080B. The opinions in this paper are those of the authors and do not necessarily reflect the views of the Navy Department or the naval service at large. Experiments reported herein were conducted according to the principles enumerated in "Guide for Laboratory Animal Facilities and Care" prepared by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences—National Research Council.

half-esterified sulfate residue. This saponin ester at the $1.0 \cdot 10^{-4}$ M level has previously been shown (Friess *et al.*, 1967) to exert powerful and irreversible blocking actions on the in vitro phrenic nerve diaphragm preparation (PN-D) of the rat, working isotonically in a normobaric environment of $O_2$ $CO_2$. The desulfated ester (DeH) is less potent and less irreversible in its actions by about 1 order of magnitude.

The present work offers an important extension of these findings to PN-D tissue toxin interaction behavior under pressurized environmental conditions, with the working tissues saturated with He $N_2$ $O_2$ $CO_2$ gas mixtures at total pressures equivalent to




| SUGAR | SYMBOL |
|---|---|
| D - GLUCOSE | G |
| D - XYLOSE | X |
| D - QUINOVOSE | Q |
| 3 - O - METHYLGLUCOSE | G - OMe |

FIG. 1. Provisional structure of the major component of Holothurin A.

200–600 ft depths of sea water. The contrast in sensitivity to the charged (holothurin, H) and uncharged (DeH) marine saponins, at depth vs at ambient pressure, furnishes some useful observables for probing details of PN-D junctional receptor function. Further, the sensitivity of PN-D preparations to H as a function of the degree of loading imposed on the muscle has also been studied, yielding surprising information relative to receptor–toxin interactions.

## METHODS

The techniques for working deployment of in vitro PN-D preparations in a hyperbaric thermostated chamber, with automated control of paired electrical stimuli to the phrenic nerve (N-twitch) and muscle (M-twitch) and external recording of twitch tensions from a chamber-mounted force transducer, have been described previously (Friess *et al.*,

1968). Rats and guinea pigs used for the preparations were selected within the weight range 185–215 g. Unfortunately, limitations on saponin supply restricted experiments with the guinea pig tissues to the muscle loading sector of the study. The programmed loading of the muscle tissue in the range 0–20 g/g of tissue was set during the control time sector of each experiment.

To meet the tissue requirements for $O_2$ and $CO_2$ at the elevated pressures of the He $N_2$ $O_2$ $CO_2$ gas mixtures used for hyperbaric saturation, specific partial pressures of these metabolically important gases were selected to assure the prolonged PN-D tissue viability (up to 45 min of working time at elevated pressures) achieved in recent work (Friess *et al.*, 1970) with He-containing atmospheres. The gas mixtures were prepared by gauge from highest purity commercial components in a precision mixing manifold, and the compositions were checked by independent analyses for $O_2$ and $CO_2$ content. The mixtures were of the composition: $He(50\%)$-$N_2(27\%)$-$O_2(20\%)$-$CO_2(3\%)$. They were employed at total saturation pressures of 130 and 330 psia, for which conditions records of extended viability of the rat PN-D preparation have previously been obtained.

The holothurin A and DeH were obtained from the Mount Sinai laboratories in analytical purity. Solutions of these agents in aqueous medium or in the bicarbonate Ringer were made freshly before use. Maintenance of aliquots of these solutions in the hyperbaric chamber for remotely controlled addition to the tissue bath at the appropriate time point during an experimental protocol, was accomplished by means of a holding funnel system calibrated for delivery and an electromagnetic valve.

In a typical experiment involving a PN-D preparation and a toxin studied under high pressure, the protocol followed the sequence:

1. The PN-D preparation was mounted in the hyperbaric chamber, its Ringer tissue bath was equilibrated at 37 C and flushed at 1 ata for 5 min with $95\%$ $O_2$ $5\%$ $CO_2$ and a series of normobaric N-twitch and M-twitch control readings were taken under the selected muscle load.

2. The chamber was then flushed with the diving gas mixture for 5 min, and compression was begun at a rate of 10 psig/min. After full pressure and temperature (37.0 ± 0.4 C) equilibration, the diaphragm was worked under electrical stimulus for 3 min with alternate stimuli to nerve and muscle at 3-sec intervals and a 10-sec spacing on the repetition cycle for each pair of twitch responses. The toxin solution was then added to the tissue bath via its storage funnel.

3. The diaphragm was then caused to work under stimulus, with automatic recording of twitch and baseline tensions, until either the N-twitch tension had been reduced to $50\%$ of its control (pressurized) level, or the preparation had worked for a period totaling 10.5 min without reaching $50\%$ reduction in twitch tension. This time period amounted to a short segment of the mean viability times under the given pressures. The bomb pressure was then released gradually over the course of 5 min.

4. The preparation was then washed repeatedly with fresh, gassed ($95\%$ $O_2$/$5\%$ $CO_2$) Ringer, equilibrated with a fresh bath of the same medium and reinserted into the bomb. The bomb and its contents were then flushed with the $O_2$ $CO_2$ gas mixture at 1000 ml/min, and the preparation was finally subjected to 5 more min of work under 1 ata of $O_2$ $CO_2$, with continuous recording of postwash response and baseline tensions during the normobaric recovery interval.

A minimum of 2 preparations was employed for each experimental point involving working exposure to a given toxin at a given concentration and pressure. For those experiments recording isometric PN-D function under saturation with 1 ata of $O_2/CO_2$, the working interval following addition of toxin to the bath was set at 5.5 min.

## RESULTS

Toxin-tissue interaction behavior as a function of environmental pressure, considering first the individual toxin effects under varying hyperbaric conditions and then the sets of contrasts between H and DeH effects under variation in pressure, forms a complex picture. It is therefore useful to establish a set of characterization parameters describing the actions of each toxin on the preparation, to permit ready comparisons as a function of environmental variables. A pattern for such a set of parameters has been suggested previously (Friess *et al.*, 1967) in terms of characterization indexes for various sections of the response vs time records. Considering a representative response vs time curve (Fig. 2) for a rat PN-D preparation at 330 psia subjected to a $1.0 \cdot 10^{-4}$ M concentration of H , when working at 10 g loading/g of tissue, the curve sectors record the

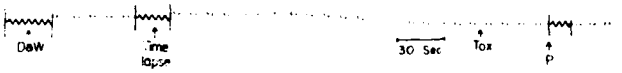



FIG. 2. Representative tracing (reading right to left) of the action of $1.0 \cdot 10^{-4}$ M H on the rat PN-D preparation at 330 psia. The symbols refer to: P, pressurization; Tox, addition of toxin to the tissue bath; D & W, depressurization and washing with bicarbonate Ringer. In each pair of twitch responses the N-twitch occurs first.

phenomena of: (1) a toxin-induced increase (upward displacement) in baseline tension; (2) progressive blockade of N- and M-twitch responses with increasing incubation time; and (3) some degree of retention of twitch response capability on depressurization and washing to remove toxin. These responses are qualitatively similar to those seen with the PN-D tissue operating isotonically at 1 ata pressure. Therefore, the same categories of characterization parameters were extracted from the toxin-tissue response curves under pressure as those previously tabulated (Friess *et al.*, 1967) for the preparation working under 1 ata of $O_2/CO_2$.

The descriptive parameters presently tabulated for the toxin-tissue pressure experiments include: (1) a measure of the mean rate of rise of baseline muscle tension as produced by H or DeH; (2) the maximum baseline tension relative to the reference M-twitch tension at the given pressure; (3) measures of twitch tension augmentation, if any; (4) the extent of reduction of N- and M-twitch tensions at given reference times after toxin addition to the bathing medium, corrected for twitch tension changes in control hyperbaric preparations; and (5) the extent of recovery of N- and M-twitch tensions toward prepressurization control levels following depressurization and removal of toxin by washing with $O_2/CO_2$ gassed Ringer medium. These parameters, for experiments with rat PN-D preparations worked isometrically at 14.7 psia in bicarbonate Ringer saturated with $O_2(95\%)/CO_2(5\%)$, and at 130 and 330 psia with the $He/N_2/O_2/CO_2$ gas mixture, are summarized beginning with Table 1. Table 1 presents

TABLE 1

ALTERATIONS IN BASELINE TENSION OF THE RAT PN-D PREPARATION SUBJECTED TO INCUBATION WITH H OR DeH AT VARIOUS PRESSURES

| Response parameter | Gas mixture | Saturation pressure (psia) | Toxin | Value[a] at given incubation concentration | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 1.0 | $10^{-5}$ M | | 1.0 | $10^{-4}$ M | | 1.0 | $10^{-3}$ M | |
| Mean rate of rise of initial contracture (mm sec M) | $O_2$ $CO_2$ | 14.7 | H | 9.3 | 3.3 | $10^2$ | 8.2 | 1.0 | $10^2$ | | | |
| | | 14.7 | DeH | | | | 1.8 | 0.3 | $10^2$ | 1.9 | $10^2$ | |
| | | 14.7[b] | H[b] | | | | 14.4 | 4.6 | $10^{2b}$ | | | |
| | | 14.7[b] | DeH[b] | | | | 2.36 | 0.24 | $10^{2b}$ | | | |
| | He $N_2$ $O_2$ $CO_2$ | 130 | H | | 0.0 | | 5.2 | 2.2 | $10^2$ | | | |
| | | 130 | DeH | | | | 7.0 | 4.1 | $10^2$ | 11.2 | $10^2$ | |
| | | 330 | H | 2.1 | 1.5 | $10^2$ | 3.5 | 3.5 | $10^2$ | | | |
| | | 330 | DeH | | | | 1.5 | 0.4 | $10^2$ | 2.4 | $10^2$ | |
| Maximum value of contracture[c] | $O_2$ $CO_2$ | 14.7 | H | | | | 72 | 16 | | | | |
| | | 14.7 | DeH | | | | 38 | 8 | | | | |
| | | 14.7[b] | H[b] | | | | 150 | 32[b] | | | | |
| | | 14.7[b] | DeH[b] | | | | 32 | 2[b] | | | | |
| | He $N_2$ $O_2$ $CO_2$ | 130 | H | | | | 40 | 14 | | | | |
| | | 130 | DeH | | | | 10 | 5 | | | | |
| | | 330 | H | | | | 25 | 7 | | | | |
| | | 330 | DeH | | | | 33 | 2 | | | | |

[a] Mean response values ± SD for preparations working in *isometric* mode, under a load of 10 g g of tissue.
[b] Mean values previously recorded (Friess *et al.*, 1967) for preparations working in *isotonic* mode.
[c] Relative to control M-twitch tension (under pressure) = 100.

TABLE 2

CHANGES IN TWITCH TENSION OF THE RAT PN-D PREPARATION SUBJECTED TO INCUBATION WITH H⁻ OR DeH AT VARIOUS PRESSURES

| Response parameter | Gas mixture | Saturation pressure (psia) | Toxin | Value[a] at given incubation concentration | | |
|---|---|---|---|---|---|---|
| | | | | $1.0 \cdot 10^{-5}$ M | $1.0 \cdot 10^{-4}$ M | $1.0 \cdot 10^{-3}$ M |
| Twitch tension augmentation (peak)[b] | | | | | | |
| N-twitch | $O_2$ $CO_2$ | 14.7 | H | | | |
| | | 14.7 | DeH | | | |
| | He $N_2$ $O_2$ $CO_2$ | 130 | H | T(120) | T(30) | |
| | | 130 | DeH | | | |
| | | 330 | H | 6 · 1(30) | T(120) | |
| | | 330 | DeH | | T(30) | T(30) |
| M-twitch | $O_2$ $CO_2$ | 14.7 | H | | | |
| | | 14.7 | DeH | | T(60) | |
| | He $N_2$ $O_2$ $CO_2$ | 130 | H | | 10 · 0(120) | |
| | | 130 | DeH | | | |
| | | 330 | H | 7 · 2(60) | T(120) | |
| | | 330 | DeH | | T(30) | T(30) |
| Twitch blockade[c] | | | | | | |
| N-twitch | $O_2$ $CO_2$ | 14.7 | H | 3 · 5(270), P | 57 · 8(330), M | |
| | | 14.7 | DeH | | 19 · 5(330), G | 50(60), P |
| | | | | 0(330) | 19 · 1(330), P | |
| | He $N_2$ $O_2$ $CO_2$ | 130 | H | | | |
| | | | | 15 · 3(450), G | 43 · 16(516), P | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | | 16 ± 2(270), G | |
| | | 130 | DeH | | | 64(258), P |
| | | | | | 24 ± 0(630), G | |
| | | | | 0(330) | | |
| | | 330 | H | | 29 ± 16(270), P | |
| | | | | 18 ± 7(630), G | | |
| | | | | | 16 ± 1(330), M | |
| | | 330 | DeH | | | 57(270), P |
| | | | | | 29 ± 10(510), M | |
| M-twitch | $O_2$ $CO_2$ | 14.7 | H | 10 ± 2(270), P | 38 ± 2(330), G | |
| | | 14.7 | DeH | | 24 ± 15(330), G | 54(60), G |
| | | | | 9 ± 0(330), G | 0(330) | |
| | $He/N_2$ $O_2$ $CO_2$ | 130 | H | | | |
| | | | | 12 ± 5(630), G | 17 ± 1(510), G | |
| | | | | | 19 ± 7(330), G | |
| | | 130 | DeH | | | 44(258), P |
| | | | | | 26 ± 7(630), G | |
| | | | | 0(300) | | |
| | | 330 | H | | 17 ± 11(270), M | |
| | | | | 27 ± 9(630), G | | |
| | | | | | 11 ± 3(270), G | |
| | | 330 | DeH | | | 23(270), M |
| | | | | | 17 ± 1(510), G | |

[a] Mean response values ± SD for preparations working in *isometric* mode, under a load of 10 g g of tissue.
[b] Mean values ± SD at the given times (sec) post-addition of toxin, vs pressurized controls. Values ± 5% are designated as trace (T).
[c] Mean values ± SD of % extent, relative to control, at times (sec) indicated. Extent of twitch tension recovery after release of pressure and washing with Ringer ($O_2$ $CO_2$, 1 ata) indicated by: P (poor), 0 – 25%; M (moderate), 25–50%; G (good), >50%.

data for the changes in PN-D response behavior relating to the rates of rise and the maximum increases in baseline tension. Also included in Table 1, for reference purposes, are data on toxin-induced baseline changes previously cited for H and dDeH actions on the PN-D preparation worked isotonically under 14.7 psia of $O_2$, $CO_2$ (Friess *et al.*, 1967). Table 2 contains the results on augmentation blockade of twitch response tensions at times early (270–330 sec) and late (500–630 sec) in the periods of incubation with toxin, together with notations on twitch blockade reversibility following depressurization and washing.

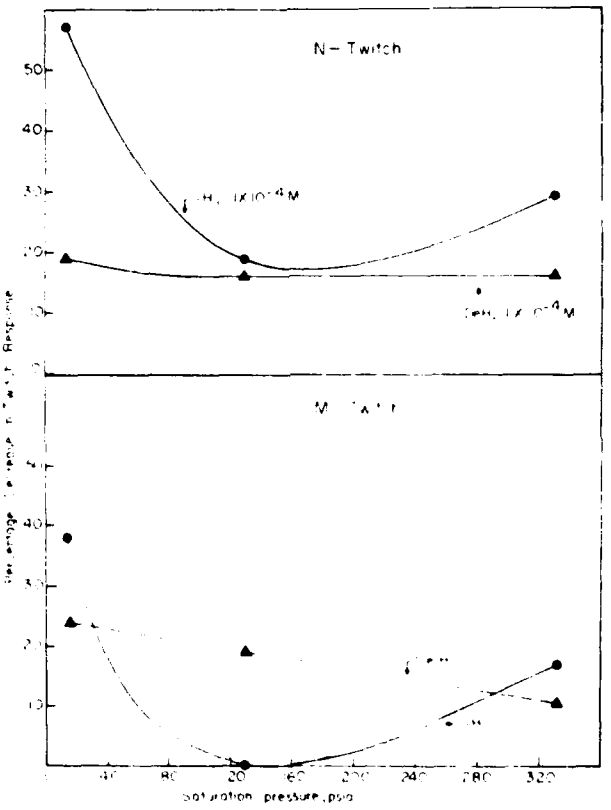



FIG. 3. Effects of change in total environmental pressure on N- and M-twitch blockade in the rat PN-D preparation. H and DeH concentrations, $1.0 \times 10^{-4}$ M, respectively; tissue loading, 10 g g; toxin incubation time, 270–330 sec.

The findings summarized in Tables 1 and 2 reveal a striking dependence of toxin-tissue effects on the environmental pressure, with the nature of the dependency also bearing a direct link to the charge character of the toxin. These points are best illustrated by the set of representative curve trends drawn from data of Tables 1 and 2 and sketched in Fig. 3.

Figure 3 records the effects of total pressure on twitch blockade indexes for N- and M-twitch responses (isometric, 10 g/g of tissue) at the reference time interval 270–330

sec following addition of saponin to the bath, for H and DeH at $1.0 \times 10^{-4}$ M, respectively. It is to be noted that the blocking potency of anionic H is very pressure-dependent in terms of effects on both N- and M-twitch responses; the saponin potency first falls sharply as the total environmental pressure is increased from 14.7 to 130 psia, and then increases moderately as the ambient pressure is further raised toward 330 psia. In the case of DeH, the effect of ambient pressure on relative potency is virtually nil for N-twitch responses and only marginal with respect to slight decreases in M-twitch tension as pressure traverses the range 14.7–330 psia.

The effects of graded muscle tension on sensitivity of PN-D tissues to functional attack by H have also been studied, using preparations from the rat and the guinea pig. The data from these experiments afford some surprises in terms of the dependence of

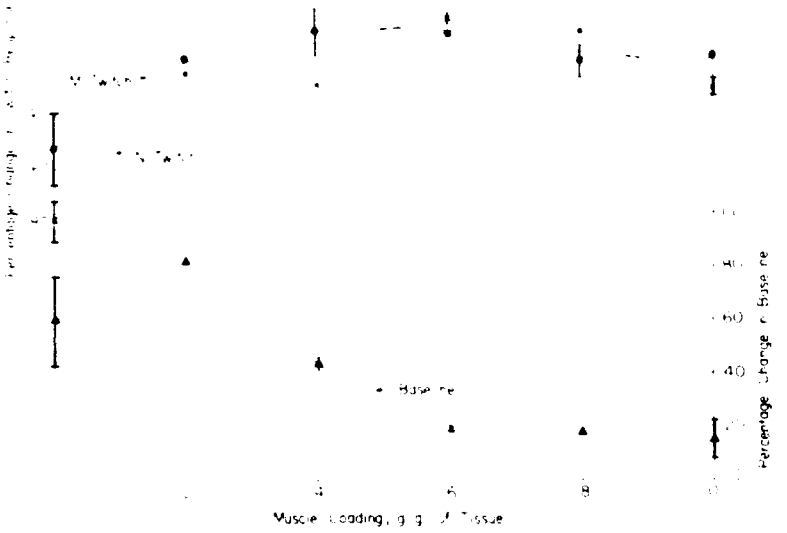



FIG. 4. Effects of muscle loading (g/g of tissue) on potency of $1.0 \times 10^{-4}$ M H in blockade of rat PN-D twitch response and on change in baseline tension. Units on baseline tension ordinate are relative to control M-twitch tension = 100.

saponin potency on the degree of muscle loading. First, from representative experiments using the rat PN-D preparation at 14.7 psia pressure ($O_2$, $CO_2$), the effects of $1.0 \times 10^{-4}$ M H on N- and M-twitch responses and baseline tension relative to presaponin control levels are shown in Fig. 4 as a function of degree of loading of the muscle. The values shown are taken at a reference time (330 sec) near the end of the saponin incubation interval. It is seen from Fig. 4 that the effectiveness of $1.0 \times 10^{-4}$ M H in depressing both N- and M-twitch responses of the rat PN-D at this reference time is quite pronounced at zero loading of the muscle, diminishes toward zero blocking potency as the loading is increased over the range 2–6 g/g of tissue, and then increases slightly as the load reaches 10 g/g of tissue. Further, the baseline tension increase produced by H is seen to be quite sensitive to the degree of muscle loading; a relatively large increase in baseline tension evident at low loading levels (0–2 g/g of tissue) gives way to progressively smaller saponin-induced increases as the muscle load approaches 8–10 g/g of tissue.

TABLE 3

EFFECTS OF HOLOTHURIN A ON RESPONSE PARAMETERS OF THE PHRENIC NERVE–DIAPHRAGM PREPARATION, RAT AND GUINEA PIG, AS A FUNCTION OF MUSCLE LOADING AND INCUBATION TIME

| Animal species | Muscle loading (g/g tissue) | Percentage changes in response parameters[a] | | | | | |
|---|---|---|---|---|---|---|---|
| | | N-Twitch | | M-Twitch | | Baseline tension | |
| | | 150 Sec | 330 Sec | 150 Sec | 330 Sec | 150 Sec | 330 Sec |
| Rat | 0 | 85 ± 7 | 69 ± 8 | 84 ± 7 | 52 ± 13 | 99 ± 11 | 60 ± 16 |
| | 2 | 47 ± 7 | 24 ± 16 | 37 ± 11 | 19 ± 20 | 150 ± 49 | 82 ± 17 |
| | 4 | 10 ± 5 | 29 ± 22 | 6 ± 4 | 9 ± 9 | 61 ± 8 | 43 ± 3 |
| | 6 | N[b] | N | N | 10 ± 10 | 41 ± 3 | 19 ± 4 |
| | 8 | N | 9 ± 5 | N | 20 ± 6 | 35 ± 7 | 17 ± 0 |
| | 10 | 8 ± 4 | 30 ± 3 | 9 ± 7 | 18 ± 15 | 26 ± 5 | 15 ± 7 |
| Guinea pig | 0 | 47 ± 30 | 66 ± 10 | 49 ± 25 | 50 ± 12 | 76 ± 43 | 84 ± 24 |
| | 4 | 25 ± 2 | 14 ± 0 | 21 ± 1 | N | 67 ± 5 | 49 ± 14 |
| | 8 | 7 ± 5 | 7 ± 7 | 8 ± 4 | N | 50 ± 16 | 36 ± 7 |
| | 12 | N | 13 ± 6 | N | N | 8 ± 2 | 14 ± 0 |
| | 16 | N | N | N | N | 10 ± 2 | 11 ± 2 |
| | 20 | 6 ± 1 | N | N | N | 22 ± 0 | 18 ± 3 |

[a] Percent changes (means ± SD) for duplicate preparations with respect to control (pretoxin) levels. HT added to the tissue bath at zero time; final concentration $1.0 \times 10^{-4}$ M.

[b] Values designated as N (negligible) amount to less than 5% changes from control levels.

The full set of data on the variation in efficacy of $1.0 \cdot 10^{-4}$ M H actions on PN-D preparations from the rat and guinea pig, as a function of muscle loading and reference time of incubation of toxin with tissue, is summarized in Table 3. The incubation times selected as representative of early and late effects on the tissues were taken at 150 and 330 sec, respectively. From Table 3 it can be seen that the generalizations drawn in discussion of Fig. 4 for the rat PN-D at 330 sec of incubation apply quite well to the effects of change in tissue loading on the potency of $1.0 \cdot 10^{-4}$ M H in either rat or guinea pig tissues, at both long and short incubation times. Pronounced toxin effects on N-twitch, M-twitch and baseline tensions found at low muscle loadings rather generally fade to negligible levels as the static loading factor is increased toward 10 g/g tissue for the rat and 20 g g tissue for the guinea pig preparation.

## DISCUSSION

The present findings on the effects of elevated environmental pressures and variation in muscle loading on working PN-D tissues, leading to perturbation of H DeH actions, offer some unexpected elements of insight into possible modes of interaction of toxin with tissue receptors. Beginning with the observations that initial increases in pressure and muscle loading cause a sharp drop in the potency of H as a twitch blocking agent, it is clear that a suitable model for toxin-sensitive receptors must be accommodated in a tissue locus which is readily susceptible to influence by external stresses imposed from

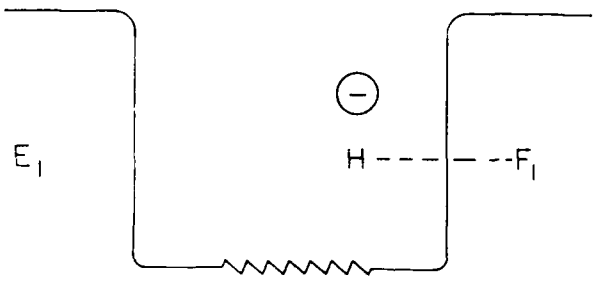



FIG. 5. Postulated crevasse model for H -sensitive membrane receptors.

the physical environment. Such a locus in the junctional area has been invoked previously (Friess *et al.*, 1965) to account for features of H DeH interactions with PN-D tissues in the form of a shallow surface "pore" or crevasse, as illustrated in Fig. 5. In this figure, $F_1$ surface sites susceptible to H attack are pictured within the interior contours of shallow depressions located below the PN-D nerve terminals on the postjunctional membrane, contiguous to pore surface areas $E_1$ characterized by binding properties similar to those of the enzyme acetylcholinesterase. For this toxin receptor model, initial distortion of the interior surfaces of a given pit or crevasse by increased environmental pressure or loading-stretching of muscle fibrils could well diminish the degree of fit of H to the $F_1$ binding loci. The further observation that blocking actions of DeH are considerably less sensitive to external pressure than those of H (Fig. 3) could in turn be accommodated by the possibility that major DeH binding occurs at or

near the top of a receptor crevasse, in a lesser spatial proximity to the interior, deformable $F_1$ surface loci. These postulations are also in accord with the previous finding (Friess *et al.*, 1967) that DeH is able to effect a considerable degree of protection of PN-D excitability against the irreversible blocking actions of $H^+$: the binding of DeH in blocking mode near the top surfaces of a receptor crevasse could well impede both free access of $H^+$ to intimate contact with $F_1$ surfaces at depth and subsequent binding-disruption at those surfaces.

Finally, a satisfactory model for $H^+$-receptor interactions in PN-D tissues should also adapt to the present finding (Fig. 3) that still higher external pressures, near 3 psia, act to restore some of the $H^+$ blocking potency that is lost at 130 psia. The crevasse receptor model of Fig. 5 is sufficiently flexible to afford a rationale for this point: continuation of the surface deformation by increase in total pressure could lead to ultimate loss of pit or crevasse structure in the $H^+$-receptor areas of the membrane and fuller functional exposure of all buried $F_1$ sites to destructive attack by $H^+$ ions from solution phase.

This alteration in PN-D sensitivity to $H^+$ under high external pressures could primarily reflect a direct perturbation on membrane surface configuration by total pressure per se, but the possibility also arises that an elevated partial pressure of $CO_2$ in the gas phase may contribute to the process of reemergence of $H^+$ sensitivity. Recalling that rat PN-D tissues display a positive in vitro requirement for $HCO_3^-$ ions (Shaw and Stadie, 1957, 1959), it is at least intriguing to note the possibility that sizable concentrations of $HCO_3^-$ ions in solution created by high gaseous $P_{CO_2}$ levels may function in part to retain the operational integrity of tissue receptor areas sensitive to attack by anionic surfactants like $H^+$. Viewed as a problem in retention of ability to gate the active fluxes of $Na^+$, $K^+$ and $Ca^{2+}$ ions at membrane-localized receptors, the negatively charged bicarbonate ion could well participate in maintenance of surface configuration at receptor loci against high pressure stresses. Protection of configuration by electrostatic binding between adjacent polar heads of membrane-bound lipid molecules might be pictured in the form of ionic complexes like $NR_4^+ \cdots HCO_3^- \cdots NR_4^+$ within pores or crevasses, contributing to resistance against deformation.

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